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A STUDY OF THE SANITARY
QUALITY OF DRINKING WATER IN RURAL DISTRICTS OF
CRAWFORD COUNTY, KANSAS

APPROVED:

A Thesis Submitted to the Graduate Division in
Partial Fulfillment of the Requirements for the Degree
of Master of Science

By

Kenneth Andrew McClure

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Pittsburg, Kansas

May, 1942

ACKNOWLEDGMENTS

The writer wishes to express his sincere appreciation to Dr. J. Ralph Wells for his kind cooperation and helpful encouragement in the

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ACKNOWLEDGMENTS

The writer wishes to express his sincere appreciation to Dr. J. Ralph Wells for his kind cooperation and helpful encouragement in the preparation of this thesis; also to the farmers of Crawford County, Kansas for their cooperation and interest taken in this work.

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ABSTRACT

This study involved the testing of seventy-six wells and thirty cisterns in representative rural districts of Crawford County, using methods specified by the American Public Health Association, eighth edition of Standard

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These findings seem to warrant the following conclusions. First, deep well water, as should be expected, is less often polluted and therefore safer than shallow wells. Second, filters and covers on cisterns should be cleaned and repaired more often. Third, there is a real need for the sanitary location and construction of wells to be used for the drinking water supplies. Fourth, the construction and location of rural out-buildings, especially privies, because of their relation to the pollution of water supplies, should receive special attention.

INTRODUCTION ABSTRACT PROBLEM

This study involved the testing of seventy-six wells and thirty cisterns in representative rural districts of Crawford County, using methods specified by the American Public Health Association in the eighth edition of Standard Methods for the Examination of Water and Sewage.

The results of the tests showed that only fourteen of the 106 wells and cisterns tested did not yield Escherichia coli. Of the ninety-two polluted drinking water supplies twenty-seven were from cisterns, while sixty-five were from wells. Of these sixty-five only five of the fourteen deep wells in the group showed evidence of contamination.

These findings seem to warrant the following conclusions. First, deep well water, as should be expected, is less often polluted and therefore safer than shallow wells. Second, filters and covers on cisterns should be cleaned and repaired more often. Third, there is a real need for the sanitary location and construction of wells to be used for the drinking water supplies. Fourth, the construction and location of rural out-buildings, especially privies, because of their relation to the pollution of water supplies, should receive special attention.

INTRODUCTION AND PROBLEM

The occasional out-break of typhoid fever in Crawford County, Kansas, the source of which might have been water, has indicated the necessity of a scientific study of the drinking water especially in the rural districts of the county.

To our knowledge, there has never been any organized research in this county to prove or disprove the contamination of the well and cistern water, which is the main source of drinking water for most rural people. Health authorities of the larger cities through necessity, have taken steps to prevent the contamination of their drinking water supplies. Pittsburg, for example, has as safe a drinking water as may be found anywhere. Some of the second and third class cities and villages of the county, however, do not have water which is so well protected.

The problem therefore, was determining the extent of pollution of well and cistern water in representative districts of Crawford County, Kansas, using the procedure specified in Standard Methods of Water Analysis.

of water supplies, and perhaps to a less degree, the interpretation of the results obtained. The laboratory procedures were brought together in the first edition of Stan-

¹John F. Norton, The Newer Knowledge of Bacteriology and Immunology, Jordan and Falk, (1928), pp. 362-364.

²Ibid.

LITERATURE

The accumulation of epidemiological evidence relating drinking water to disease began about the middle of the last century. With the establishment of the germ theory of disease it is hardly surprising to find that some of the early bacteriologists were interested in the micro-organic content of waters of all characters and to find them offering biological methods to detect dangerous contamination of water supplies. Emmerich,¹ in 1878, proposed the subcutaneous injection of water, or of extracts of residues left after evaporation, into rabbits. A water dangerous to health was supposed to produce a rise in temperature in animals and subsequent death. Koch² suggested a gelatin-culture method for determining bacteria in water as early as 1881, but it was many years before bacteriological methods were sufficiently perfected to be of practical value.

Between 1892 and 1900 bacteriological technique was developed to an extent to warrant some general agreement among bacteriologists in regard to methods for the testing of water supplies, and perhaps to a less degree, the interpretation of the results obtained. The laboratory procedures were brought together in the first edition of Stan-

¹John F. Norton, The Newer Knowledge of Bacteriology and Immunology, Jordan and Falk, (1928), pp. 362-364.

²Ibid.

dard Methods of Water Analysis by the American Public Health Association (1926). While the periodic publication of these standard procedures establishes reasonably satisfactory routine methods for water analysis, these methods cannot and should not be regarded as terminating all problems of sanitary bacteriological water analysis. A similar statement might be made concerning the establishment of standards for the purity of water supplies, a necessary and useful procedure but one which must be constantly subject to revision.

Perry³ of the Maryland State Department of Health, pointed out that while the coliform group of bacteria offers a satisfactory test to determine the probable freedom of drinking water from ordinary pathogenic bacteria of the enteric group, the use of one of the simpler tests such as brilliant-green lactose bile medium, would obviate the need for confirmation, would be sufficiently accurate and sensitive for general purposes, and would make results available earlier.

The time saved could be profitably used, when necessary, for the determination of Escherichia coli. Information on Escherichia coli pollution would be of considerable value in many small drinking water supplies which it is not practical to treat or where treatment is not warranted on the basis of sanitary inspection.

He further pointed out that however valid the test for

³C. A. Perry, "Panel Discussion of Coliform Bacteria," Jour. of Bact., 36: 451. 1938

the coliform group may be for drinking water which may be filtered, chlorinated or protected, the use of the group has not been found satisfactory for estimating pollution in shell fish in which certain coliform bacteria, especially of the *Aerobacter Cloacae* type, have a natural habitat and grow to large numbers under suitable temperatures and in the absence of pollution.

France⁴ (1932) made a study to investigate the adequacy of the routine procedure of the Standard Methods of Water Analysis for bacteriological examination of water when applied to privately owned rural water supplies.

Strains of Escherichia coli were isolated from 172 samples of water which had been examined in the laboratory and condemned by the Standard Methods procedure as unfit for drinking purposes. A total of 223 strains were isolated and confirmed as Escherichia coli by the presumptive test, the partially confirmed test and the completed test of Standard Methods of Water Analysis. In addition to the strains taken from water, he also isolated 178 strains from feces and compared the two groups. All strains from water and from feces were studied for their reactions to the methyl red, Voges-Proskauer, sodium citrate, uric acid tests, and for indol production in an effort to differentiate the strains of Escherichia coli of the fecal type if possible.

⁴Ralph L. France, "Studies of Bacterium coli in Privately Owned Rural Water Supplies," Jour. of Bact., 25: 623. 1933

The results of the work showed that the dependence of the Standard Methods procedure alone for determining the sanitary quality of drinking water, especially from privately owned supplies of unknown history, results in too many of the samples being condemned.⁵ The need of a supplementary differential test was indicated but he felt that his results did not justify the recommendation of any particular test or group of tests.

In honor of the Cherokee Indians, Cherokee County was divided by the legislature into two parts with Bourbon County receiving a portion off the north. The remaining section was divided into two equal parts with the southern section being called Cherokee County, while the northern portion was named Crawford County.⁶ This left Crawford County with an aggregate of nearly 587 square miles.

John Hathaway was the first known settler in the county. Mr. Hathaway settled in the northeast section known as Lincoln Township. This was in 1844. In 1848, John Lemon settled in Osage Township near a village now known as Mondaville. Sometime later a man named Smear built the first log cabin in Baker Township. By 1852 there were about five families living in Lincoln Township. Sherman Township claimed a settler by the name of Matthews in 1850. In 1861, Buchanan, then President of the United States, sent soldiers into the "Neutral Lands" to drive out the settlers by burning their

⁶ Crawford County was named after S. J. Crawford who was Governor of Kansas when the county was established.

⁵Ibid

CRAWFORD COUNTY, KANSAS

History

The origin of Crawford County, Kansas may be traced to McGee County which included what is now Cherokee and Crawford Counties. Prior to 1860 this section was known as McGee County. In 1860 the name was changed from McGee County to Cherokee County in honor of the Cherokee Indians. Cherokee County was divided by the legislature into two parts with Bourbon County receiving a portion off the north. The remaining section was divided into two equal parts with the southern section being called Cherokee County, while the northern portion was named Crawford County.⁶ This left Crawford County with an aggregate of nearly 587 square miles.

John Hathaway was the first known settler in the county. Mr. Hathaway settled in the northeast section known as Lincoln Township. This was in 1844. In 1848, John Lemon settled in Osage Township near a village now known as Monmouth. Sometime later a man named Smear built the first log cabin in Baker Township. By 1852 there were about five families living in Lincoln Township. Sherman Township claimed a settler by the name of Matthews in 1850. In 1861, Buchanan, then President of the United States, sent soldiers into the "Neutral Lands" to drive out the settlers by burning their

⁶Crawford County was named after S. J. Crawford who was Governor of Kansas when the county was established.

homes and destroying their crops. However, other settlers soon moved into the section.⁷

The source of drinking water for nearly all of these families was found in shallow wells which they had constructed.

Crawford County has since grown in population until at one time it was the fourth ranking county in the state. At present, Crawford County ranks fifth in population in Kansas. According to the figures in 1941 the county had 45,027 inhabitants including the city of Pittsburg. The present population of Pittsburg is near 18,162. Girard is the second largest city in the county. The census of 1941 showed this community to have 2,598 persons. The rural communities⁸ of Hepler, McCune, Walnut, Cherokee, Arma, Mulberry and Arcadia, as well as Frontenac range in population from 275 to 1,194.

Crawfordsville, now non-existent, was the first town in the county, being located two miles west of what is now Girard. One of the first wells of the county was constructed here prior to 1865. It is estimated that this well is at least seventy-seven years old. In McCune, while wrecking a fifty-seven-year-old building in 1941, workers discovered an old well. This well was located under the rear of the building and was estimated to be nearly seventy years old. It was determined that this well was last used sometime in 1883.

⁷Kansas History, A Cyclopedia of Kansas History, I, p. 471.

⁸The McCune Herald, (June 20, 1941)

Under the Roesse Drug Store in McCune is another ancient well said to be almost sixty years old. The water in this well is still being used.

The present condition of some of rural and small town drinking water supplies of this county should be viewed with alarm. Due to the construction of government defense plants in this area there has been an influx of families, the heads of which are working in these plants. Many of these families have settled in the smaller communities. This increase in population has created a serious problem in the disposal of sewage because of the danger of polluting the drinking water supply. One large shell loading plant located about nine miles west of McCune, Kansas, has caused the population there to double during the last six months. These incoming families are causing health authorities considerable concern. Thus, McCune is faced with the dire need of a sewage system because of the possible danger to drinking water pollution.

Upon investigation very few sanitary toilets are to be found. Cess pools and septic tanks, due to increased usage, are in deplorable condition. Sewage overflow outlets from these tanks are allowed to flow into the gutters of the streets. In an effort to remedy this situation, the authorities have applied for Federal funds with which to construct a modern sewage system, but, thus far have failed.

Physical Characteristics

In Crawford County there are about 350 miles of streams and 587 square miles of land. There are nine large streams in the county but no rivers. The nearest river is the Neosho, located very near the western boundary of the county.

In Walnut Township there are two creeks, the Little Walnut and the Walnut. Grant Township has only one creek, the headwaters of Hickory Creek being located there. Osage Township has two large creeks, the Hickory and the Lightning. Lightning being the largest of the two and probably the largest creek in the county, it heads in the northern part of the county. Lightning creek runs through Sherman, Crawford, Sheridan and Osage Townships. Sheridan Township boasts two sizeable creeks in the Thunderbolt and the Limestone. In Lincoln Township one will find Drywood and Cox creeks. In Washington Township we find the source of Cow creek which also flows through Baker Township. Cow Creek is one of the most notorious creeks in the county, overflowing quite easily and receding equally as quickly.

The lowest point in the county is found in the southwestern section. Not infrequently, during the rainy season, much of this section is flooded. This tends to contaminate drinking water.

Climatic Conditions

Crawford County, Kansas, is located very near the geographical center of the United States. There are sudden changes in temperature and rainfall in this county. There is comparatively rapid wind movement traveling from southwest to northeast direction. Most of the moisture which falls on the county comes from the southwest. Occasionally cyclonic winds develop and over a period of years there have been a few tornadoes. Quite frequently, however, high winds do some damage in the county especially in the section located in the southern portions. On the whole, the climate is temperate.

The temperature ranges from ten degrees below zero in the winter months to 108 degrees above in the summer. Generally, the winter temperature ranges in the lower twenties, while in the summer about eighty degrees is the median.

Hardly a year passes without the flooding of the many creeks and their tributaries in the southern townships due to excess rainfall. May leads the months in amount of rainfall. The normal monthly rainfall during this month is approximately six inches. June, also, is one of the leading months in regard to amount of rainfall. September and March, too, have their share of rain usually averaging about four inches. The average annual precipitation of the county is about forty-two inches.

METHOD AND MATERIAL

In running the tests connected with this problem methods were selected for the bacterial examination of drinking water as found in the eighth edition of Standard Methods for the Examination of Water and Sewage, published by the Office of the American Public Health Association.

Areas Sampled: It will be recalled in an earlier section of this work it was stated that Crawford County covers an aggregate of 587 square miles, and that this territory is divided into nine townships each comprising about eighty square miles. In-as-much as Crawford County is nearly a square and since the townships are similar in shape, the quadrat method of survey seemed perfectly logical, although this method was not followed entirely. The major areas tested included Osage, Baker, Crawford, Walnut, and Lincoln Townships. The minor areas from which samples were taken were Sheridan, Sherman, and Washington Townships.

Samples: In collecting the samples it was found that different methods were necessary. Some wells and cisterns had pumps, while from others the water was drawn by bucket. In taking the samples the collection was usually made from the mouth of a pump or directly from the bucket.

Because of the changes which may take place in bacterial flora on standing at ordinary temperatures, all samples which could not be run immediately were placed in the refrigerator

at a temperature of about thirty-eight degrees fahrenheit. At no time did we allow the sample to remain in the refrigerator longer than twelve hours before testing.

The bottles used were those of the wide mouth type, having been sterilized in the laboratory before seeking the samples.

After collection the sample was taken to the laboratory and preparations were made to test the drinking water for the possible contamination of Escherichia coli, and, to determine the colony count.

Dilutions: The first step was to shake the bottle containing the sample vigorously twenty-five times before preparing the dilutions. In making the dilutions one cubic centimeter of the sample was withdrawn aseptically by the use of a one cubic centimeter pipette and adding to the proper dilution tube. That is, one cubic centimeter of the original sample was placed in a tube containing nine cubic centimeters of the sterilized tap water which had been previously prepared. After mixing this first dilution tube, one cubic centimeter was with-drawn and mixed in another tube containing nine cubic centimeters of the sterilized water. This second tube then contained a 1/100 dilution of the original sample. The procedure was repeated using the 1/100 dilution and placing one cubic centimeter from that dilution into another tube, thus, making this last tube 1/1000 dilutions of the original sample. The same procedure was followed on all samples of water brought into the laboratory to be tested.

Plating: After getting the original sample into the proper dilutions, plating was the next step. This was done immediately after the dilutions were made. In preparing the plates, one cubic centimeter of the original sample was placed in each of two Petri dishes and then adding ten cubic centimeters of liquefied agar medium at a temperature of about forty-two degrees centigrade. The cover of the Petri dish was lifted just enough for the introduction of the culture medium. The lips of all test tubes and pipettes were flamed before this procedure. The medium containing the sample was mixed by slowly rotating the Petri dish, then allowed to solidify. All dilutions were handled in the same manner. After solidification, the Petri dishes were placed in the incubator at a temperature of 37.5° centigrade for twenty-four hours.

Counting: This was done with a lens giving a magnification of approximately two and one-half diameters. In this procedure the counting was made on the colonies which were distinct. The method of recording the colony counts is specified in Standard Methods for the Examination for Water and Sewage as follows:

In order to avoid fictitious accuracy and yet to express the numerical results by a method consistent with the precision of the work, the number of colonies of bacteria per ml. shall be recorded as follows:⁹

⁹Standard Methods for the Examination of Water and Sewage, eighth edition, 1936, p. 209.

Number of bacteria per ml.

From	1	to	50	shall be recorded as found					
"	51	"	100	"	"	"	to the nearest	5	
"	101	"	250	"	"	"	"	"	10
"	251	"	500	"	"	"	"	"	25
"	501	"	1,000	"	"	"	"	"	50
"	1,001	"	10,000	"	"	"	"	"	100
"	10,001	"	50,000	"	"	"	"	"	500
"	50,001	"	100,000	"	"	"	"	"	1,000
"	100,001	"	500,000	"	"	"	"	"	10,000
"	500,001	"	1,000,000	"	"	"	"	"	50,000
"	1,000,001	"	10,000,000	"	"	"	"	"	100,000

The standard test for the coli-aerogenes group includes three procedures; the presumptive test, the confirmed test, and the completed test. In this study, the work was carried beyond the completed test, as this work was to show whether the organisms were of the fecal or the non-fecal strains. In carrying out this procedure it was essential to add the indol, methyl red, Voges-Proskauer, and the sodium citrate tests.

The Presumptive Test for Escherichia coli: In the presumptive test lactose broth showing a pH of from 6.4 to 7.0 was recommended. About twenty cubic centimeters of the lactose medium was added to each of three large fermentation tubes containing vials. Ten cubic centimeters of the same medium was placed into each of several small tubes with vials.

The testing procedure included placing ten cubic centimeters of the original sample in each of three large lactose fermentation tubes containing the inverted vials, and one cubic centimeter of the original into each of three small tubes containing vials. For the other dilutions only the small tubes were used. These received aseptically one cubic centimeter of the original in each of three tubes, one cubic centimeter of

of the 1/10 dilutions into each of three tubes, same volume from the 1/100 dilutions into each of three tubes, and an equal amount from the 1/1000 dilutions, went into each of three lactose fermentation tubes.

The inoculated tubes were placed in the incubator for a period of twenty-four hours at a temperature of 37.5° centigrade. If, at the end of the twenty-four hours, none of the tubes showed gas production in any amount in the inverted vials, the tubes were incubated an additional twenty-four hours. If at the close of this incubation period, no gas appeared in any of the tubes, the test was considered negative. However, formation of gas within any of the lactose fermentation tubes during the incubation period constituted a positive presumptive test.

The Confirmed Test: If gas appeared in any of the tubes at any time during the forty-eight hour incubation period a loopful of the material from the tube showing gas in the smallest amount of water used was streaked on each of two eosin methylene blue plates. These plates were incubated at 37.5° centigrade for twenty-four hours. The results of this test were either typical or atypical colonies growing on the medium within the Petri dish. If typical colonies developed upon either plate within this period, this confirmed test was considered positive.

The Completed Test: From the eosin methylene blue plates a typical colony was selected, half of which was streaked on an

agar slant and incubated until growth appeared, while the other half of the colony was inoculated into a small lactose tube which contained an inverted vial. This tube was also incubated at 37.5° centigrade for twenty-four hours.

At the close of this incubation period if gas was found in the fermentation tube and growth on the agar slant was proved to be Gram negative non-spore forming sluggishly motile rods, the test was considered as complete and the presence of Escherichia coli was assumed.

Any deviation in the above results was probably due to contamination and the tests were re-run. In re-running the tests it was necessary to restreak an eosin methylene blue plate from the large lactose tube used in the presumptive test. This procedure was repeated until the organism was isolated in a pure culture.

After establishing the identity of Escherichia coli the next step was to differentiate the organism into fecal and non-fecal groups by the indol, methyl red, Voges-Proskauer, and sodium citrate tests.

The Indol Test: In preparing the medium for the indol test 1000 cubic centimeters of distilled water was added to ten grams of Bacto-tryptone and heated, stirring to obtain complete solution. The medium was then distributed in ten cubic centimeter portions into test tubes and sterilized at fifteen pounds, fifteen minutes at 120° centigrade.

The test reagent was made by dissolving five grams of C. P. para dimethyl-amino benzaldehyde in seventy-five cubic

centimeters of amyl alcohol and adding twenty-five cubic centimeters of concentrated hydrochloric acid. Laboratory reagent amyl alcohol was recommended. This reagent had a yellow color.

After preparation of the medium and the reagent the next step was to inoculate five cubic centimeters of the medium and incubate at 37.5° centigrade for twenty-four hours. Then, three drops of the reagent was added and mixed well. After allowing the tube to stand for about a minute the result was observed in one of two ways; a dark red color in the amyl alcohol surface layer showed a positive indol test, while the original yellow color of the reagent found on the surface showed a negative test.

The Methyl Red Test: In preparing the peptone medium for this test we added five grams of Proteose-peptone, Difco, five grams of C. P. dextrose, and five grams of dipotassium hydrogen phosphate (K_2HPO_4) to eighty cubic centimeters of distilled water. A dilute solution of the K_2HPO_4 gave a distinct pink color with phenol-phthalein. This solution was heated over steam for twenty minutes, then filtered through folded filter paper, cooled to twenty degrees centigrade, and diluted to 1000 cubic centimeters with distilled water.

The medium was distributed in test tubes placing five cubic centimeters in some tubes, ten cubic centimeters in others and sterilized. The five cubic centimeter tubes were to be used for the Voges-Proskauer test while the ten cubic

centimeter tubes were used for the methyl red test. The medium was sterilized by the intermittent method for twenty minutes on three successive days.

Indicator Solution for the Methyl Red Test: For this reagent we dissolved 0.1 gram of methyl red in 300 cubic centimeters of alcohol and diluted to 500 cubic centimeters with distilled water.

Then, ten cubic centimeter portions of the medium were inoculated from the growth on the agar slant which had been previously prepared in the completed test. The tubes were incubated at 37.5° centigrade for three days. At the close of the incubation period, five drops of the indicator solution were added to the medium. Results for a positive methyl red test showed a distinct red color in the amyl alcohol surface layer, while no change or a yellow color in the surface layer was recorded as negative.

The Voges-Proskauer Test: This test was made using the five cubic centimeter portions of the medium prepared in the methyl red test. This medium was inoculated from some of the culture growth from the same agar slant used in the methyl red test. This tube was incubated at 37.5° centigrade for twenty-four hours. At the completion of the incubation period several drops of ten per cent solution of potassium hydroxide was added; if an eosin pink color was noted in the medium the result was recorded as positive. No color in the medium constituted a negative test.

The Sodium Citrate Test: To prepare the medium for these tests there was dissolved 1.5 grams of sodium ammonium phosphate, one gram of potassium dihydrogen phosphate, 0.2 gram of magnesium sulfate, and 2.5 grams of sodium citrate (crystals) in one liter of distilled water. Then this medium was distributed into tubes in five cubic centimeter amounts and sterilized at fifteen pounds, 120° centigrade, fifteen minutes.

The medium was then inoculated with some of the growth from the agar slant which had been used in previous tests. After incubating at 37.5° centigrade for seventy-two hours, the broth was checked for growth. Growth was recorded as positive, no growth, negative.

The number of wells and cisterns including city drinking water supplies tested totalled 106. As has been previously stated, the quadrat method of survey was used as much as possible. Samples were taken from eight of the nine townships of the county. Ten samples were collected from Walnut Township, fourteen from Lincoln Township, twenty-four each from Baker and Osage Townships, twenty-two were from Crawford, ten from Washington, and one each from Sherman and Sheridan Townships.

Of the 106 samples collected, seventy-six were from wells, and thirty from cisterns. Of this total six city water supplies, Frontenac, Mulberry, Arcadia, Girard, Arma, and McCune were taken. A sample was also collected from the town pump located at Hepler.

RESULTS AND DISCUSSION

To the layman the results of this survey may be surprising, but, to the technician accustomed to doing water analysis they are not. Perhaps a survey in any rural district would yield similar results.

In discussing the results of this investigation, it seems feasible to organize them under the following headings; total colony counts, Escherichia coli tests, and the special tests.

The number and percent of the samples showing various colony counts are found in the following table, (Table I).

Cisterns	2	6	101-500
Wells	3	4	501-1000
Cisterns	5	19	1001-10000
Wells	10	14	1001-10000
Cisterns	2	6	10001-50000
Wells	2	3	10001-50000

The above table reveals that there were no cisterns where the colony count was recorded as zero. However, two wells showed no colonies. It was also interesting to discover that of the fourteen deep wells tested, not one had a colony count above fifty. It is also noted that in this work, cisterns had a higher per cent of contamination than wells (the exception in this table is in the 1-50 colonies).

It is evident in the above data that forty (53%) of the seventy-six wells, had a count of less than fifty; where-as, only twelve (40%) of the thirty cisterns had a similar count.

TABLE I.
The Number and Per Cent of Samples Showing Various Colony Counts

Source of Sample	Number of Samples	Per cent	Colony Count
Cisterns	0	0	0
Wells	2	3	0
Cisterns	12	40	1-50
Wells	38	54	1-50
Cisterns	4	13	51-100
Wells	7	10	51-100
Cisterns	5	19	101-500
Wells	14	19	101-500
Cisterns	2	6	501-1000
Wells	3	4	501-1000
Cisterns	5	19	1001-10000
Wells	10	14	1001-10000
Cisterns	2	6	10001-50000
Wells	2	3	10001-50000

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It is evident in the above data that forty (53%) of the seventy-six wells, had a count of less than fifty; where-as, only twelve (40%) of the thirty cisterns had a similar count.

The exact colony count results for each of the wells and cisterns are shown in the following table, (Table II).

TABLE II

The Source, Date, Location and Total Colony Count of all Samples Tested

Sample No.	Date	Township	Source	Colonies
1	7-22-40	Osage	S. Well	650
2	"	"	"	1600
3	7-23-40	Washington	Cistern	14
4	7-24-40	Osage	D. Well	5
5	7-29-40	"	Cistern	10
6	"	"	S. Well	450
7	7-30-40	"	Cistern	25
8	"	"	S. Well	300
9	8-1-40	"	"	65
10	"	Baker	Cistern	80
11	8-2-40	Sheridan	"	5
12	8-7-40	Osage	"	65
13	6-12-41	"	"	550
14	"	"	"	1100
15	6-16-41	"	S. Well	1700
16	"	"	"	100
17	6-17-41	"	"	500
18	"	"	"	900
19	6-18-41	"	"	85
20	"	Baker	"	1300
21	6-22-41	Osage	"	160
22	"	Baker	"	10
23	"	"	"	3
24	"	"	"	50
25	"	"	Cistern	80
26	"	"	S. Well	3500
27	6-25-41	"	"	60
28	"	"	"	50
29	"	"	"	10
30	"	"	"	1

TABLE II (cont).

Sample No.	Date	Township	Source	Colonies
31	6-25-41	Baker	Cistern	85
32	"	"	S. Well	150
33	6-26-41	Osage	"	1200
34	6-30-41	"	Cistern	550
35	"	Baker	"	1200
36	"	"	S. Well	130
37	"	"	Cistern	1100
38	"	"	"	200
39	"	"	S. Well	20000
40	"	"	Cistern	200
41	"	"	S. Well	130
42	7-8-41	Lincoln	"	110
43	"	"	"	5000
44	"	"	"	50
45	"	"	"	100
46	"	"	"	50
47	"	"	"	4500
48	"	"	Cistern	5
49	"	"	S. Well	100
50	"	"	"	11
51	"	"	"	5
52	"	Washington	"	300
53	"	"	"	5
54	"	"	D. Well	48
55	7-10-41	Crawford	S. Well	950
56	"	"	"	2000
57	"	"	"	4
58	"	"	"	2000
59	"	"	"	7
60	"	"	"	100
61	"	"	"	9500
62	"	"	Cistern	7
63	"	"	S. Well	11
64	"	"	"	10
65	"	"	D. Well	5

TABLE II (cont).

Sample No.	Date	Township	Source	Colonies
66	7-10-41	Crawford	D. Well	8
67	"	Washington	"	20
68	7-15-41	Crawford	S. Well	120
69	"	"	Cistern	500
70	"	"	D. Well	4
71	"	"	Cistern	1600
72	"	"	D. Well	20
73	"	"	S. Well	25
74	"	"	Cistern	48
75	"	"	"	10
76	"	"	S. Well	50
77	"	"	"	10
78	"	Baker	"	300
79	"	"	"	300
80	7-17-41	Osage	"	5
81	"	"	"	100
82	"	"	"	5000
83	"	Walnut	"	33
84	"	"	D. Well	20
85	"	"	"	15
86	"	"	S. Well	5
87	"	"	"	4
88	"	"	D. Well	6
89	"	"	"	5
90	"	"	S. Well	50
91	"	"	"	20
92	"	"	Cistern	20
93	"	"	S. Well	5
94	"	Sherman	Cistern	5
95	7-19-41	Osage	D. Well	5
96	"	"	Cistern	120
97	"	Baker	S. Well	500
98	"	"	Cistern	30000
99	"	Washington	D. Well	5
100	"	Lincoln	"	0

TABLE II (cont).

Sample No.	Date	Township	Source	Colonies
101	7-19-41	Lincoln	Cistern	2000
102	"	"	"	150
103	"	"	"	5
104	"	Washington	"	5
105	"	"	S. Well	0
106	"	"	Cistern	10000

LEGEND:

S. Well--Shallow Well

D. Well--Deep Well

The colony count of this group of wells and cisterns ranged from zero to 30000 colonies per cubic centimeter of water tested. It was significant to note that two wells showed a colony count of zero, while no cisterns were included in this count. The highest colony count was a cistern having 30000. The highest count among the wells was 20000. As previously stated, the counts of the wells were much lower than those of the cisterns.

As should be expected the colony count of the deep wells were comparatively low, in fact, not one of the deep wells showed a colony count above forty-eight.

The cisterns were especially high. This is probably due to uncleaned filters or no filters whatever.

The Escherichia coli tests include the presumptive, confirmed, and the completed tests. The results of these are shown in the following table, (Table III).

TABLE III
Escherichia coli Tests

Sample No.	Presumptive	Confirmed	Completed
1	Positive	Positive	Positive
2	"	"	"
3	"	"	"
4	"	"	"
5	Negative	"	"
6	Positive	Positive	Positive
7	"	"	"
8	"	"	"
9	"	"	"
10	"	"	"
11	Negative	"	"
12	Positive	Positive	Positive
13	Negative	"	"
14	Positive	Positive	Positive
15	"	"	"
16	"	"	"
17	"	"	"
18	"	"	"
19	"	"	"
20	"	"	"
21	"	"	"
22	"	"	"
23	Negative	"	"
24	Negative	Positive	Positive
25	Positive	Positive	Positive
26	"	"	"
27	Negative	"	"
28	Positive	Positive	Positive
29	"	"	"
30	Negative	"	"
31	Positive	Positive	Positive
32	"	"	"
33	"	"	"
34	"	"	"
35	"	"	"

TABLE III (cont).

Sample No.	Presumptive	Confirmed	Completed
36	Positive	Positive	Positive
37	"	"	"
38	"	"	"
39	"	"	"
40	"	"	"
41	"	"	"
42	"	"	"
43	"	"	"
44	"	"	"
45	Negative		
46	Positive	Positive	Positive
47	"	"	"
48	"	"	"
49	"	"	"
50	"	"	"
51	"	"	"
52	"	"	"
53	Negative		
54	Positive	Positive	Positive
55	"	"	"
56	"	"	"
57	"	"	"
58	"	"	"
59	"	"	"
60	"	"	"
61	"	"	"
62	"	"	"
63	Negative		
64	Positive	Positive	Positive
65	"	"	"
66	"	"	"
67	Negative		
68	Positive	Positive	Positive
69	"	"	"
70	"	"	"

TABLE III (cont).

Sample No.	Presumptive	Confirmed	Completed
71	Positive	Positive	Positive
72	"	"	"
73	"	"	"
74	"	"	"
75	"	"	"
76	"	"	"
77	"	"	"
78	"	"	"
79	"	"	"
80	"	"	"
81	"	"	"
82	"	"	"
83	"	"	"
84	"	"	"
85	"	"	"
86	"	"	"
87	"	"	"
88	Negative		
89	Negative		
90	Positive	Positive	Positive
91	"	"	"
92	"	"	"
93	"	"	"
94	"	"	"
95	"	"	"
96	"	"	"
97	"	"	"
98	"	"	"
99	Negative		
100	Negative		
101	Positive	Positive	Positive
102	"	"	"
103	Negative		
104	Negative		
105	Negative		
106	Positive	Positive	Positive

The significant fact shown by Table III is, that all samples which showed a positive presumptive reaction contained Escherichia coli when carried through the completed test.

51137 This table also shows that only fourteen of the original one-hundred-six samples collected and tested for Escherichia coli strains, were shown to contain no lactose fermenting organisms in the water. In other words only thirteen per cent showed a negative reaction to the presumptive test. It is further shown that three (10%) of the thirty cisterns tested contained no lactose fermenting organisms, and that eleven (14½%) of the seventy-six wells tested reacted negatively in the presumptive test. Of the eleven wells which were negative in the presumptive test five (49%) were from deep well sources. This would indicate that water from deeper sources would be much more safe for drinking purposes than water from any other natural source.

In classifying the organisms isolated from the completed tests into fecal and non-fecal groups it was necessary to run the samples through the special tests. These special tests included reactions to indol, methyl red, Voges-Proskauer, and sodium citrate. The results of these tests are given in the following table, (Table IV). In this table the number of the strains correspond to the number of the sample from which it was isolated.

TABLE IV

Results of Special Tests of Strains of *Escherichia coli*

Sample No.	Indol	M.R.	VP.	Cit.	Usually	Non-fecal	Fecal
1	Pos.	Pos.	Neg.	Neg.	E. coli	---	Yes
2	Neg.	"	"	"	"	Yes	---
3	"	"	"	"	"	"	---
4	Pos.	"	"	"	"	"	Yes
5	"	"	"	"	"	---	"
6	"	"	"	"	"	---	"
7	Neg.	"	"	"	"	Yes	---
8	Pos.	"	"	"	"	---	Yes
9	Neg.	"	"	"	"	Yes	---
10	"	"	"	"	"	"	---
12	Pos.	"	"	"	"	---	Yes
13	Neg.	"	"	"	"	Yes	---
14	Pos.	"	"	"	"	---	Yes
15	Neg.	"	"	"	"	Yes	---
16	"	"	"	"	"	"	---
17	"	"	"	"	"	"	---
18	Pos.	"	"	"	"	"	Yes
19	Neg.	"	"	"	"	Yes	---
20	Pos.	"	"	"	"	---	Yes
21	"	"	"	"	"	---	"
22	"	"	"	"	"	---	"
23	"	"	"	"	"	---	"
25	Neg.	"	"	"	"	Yes	---
26	"	"	"	"	"	"	---
27	"	"	"	"	"	"	---
28	"	"	"	"	"	"	---
29	"	"	"	"	"	"	---
31	"	"	"	"	"	"	---
32	"	"	"	"	"	"	---
33	"	"	"	"	"	"	---
34	Pos.	"	"	"	"	---	Yes
35	Neg.	"	"	"	"	Yes	---
36	"	"	"	"	"	"	---
37	Pos.	"	"	"	"	---	Yes
38	Neg.	"	"	"	"	Yes	---
39	Pos.	"	"	"	"	---	Yes
40	Neg.	"	"	"	"	Yes	---

TABLE IV (cont).

Sample No.	Indol	M.R.	VP.	Cit.	Usually	Non-fecal	Fecal
41	Pos.	Pos.	Neg.	Neg.	E. coli	---	Yes
42	"	"	"	"	"	---	"
43	"	"	"	"	"	---	"
44	"	"	"	"	"	---	"
46	"	"	"	"	"	---	"
47	"	"	"	"	"	---	"
48	Neg.	"	"	"	"	Yes	---
49	"	"	"	"	"	"	---
50	"	"	"	"	"	"	---
51	Pos.	"	"	"	"	---	Yes
52	Neg.	"	"	"	"	Yes	---
54	Pos.	"	"	"	"	---	Yes
55	"	"	"	"	"	---	"
56	Neg.	"	"	"	"	Yes	---
57	Pos.	"	"	"	"	---	Yes
58	Neg.	"	"	"	"	Yes	---
59	Pos.	"	"	"	"	---	Yes
60	"	"	"	"	"	Yes	---
61	"	"	"	"	"	---	Yes
62	Neg.	"	"	"	"	Yes	---
64	"	"	"	"	"	"	---
65	"	"	"	"	"	"	---
66	"	"	"	"	"	"	---
68	"	"	"	"	"	"	---
69	"	"	"	"	"	"	---
70	"	"	"	"	"	"	---
71	Pos.	"	"	"	"	---	Yes
72	"	"	"	"	"	---	"
73	Neg.	"	"	"	"	Yes	---
74	"	"	"	"	"	"	---
75	"	"	"	"	"	"	---
76	Pos.	"	"	"	"	---	Yes
77	"	"	"	"	"	---	Yes
78	Neg.	"	"	"	"	Yes	---
79	"	"	"	"	"	"	---
80	"	"	"	"	"	"	---

TABLE IV (cont).

Sample No.	Indol	M.R.	VP.	Cit.	Usually	Non-fecal	Fecal
81	Neg.	Pos.	Neg.	Neg.	E. coli	Yes	---
82	Pos.	"	"	"	"	---	Yes
83	"	"	"	"	"	---	Yes
84	"	"	"	"	"	---	Yes
85	"	"	"	"	"	---	"
86	"	"	"	"	"	---	"
87	Neg.	"	"	"	"	Yes	---
90	"	"	"	"	"	"	---
91	"	"	"	"	"	"	---
92	"	"	"	"	"	"	---
93	Pos.	"	"	"	"	---	Yes
94	"	"	"	"	"	---	"
95	"	"	"	"	"	---	"
96	Neg.	"	"	"	"	Yes	---
97	"	"	"	"	"	"	---
98	"	"	"	"	"	"	---
101	Pos.	"	"	"	"	---	Yes
102	Neg.	"	"	"	"	Yes	---
106	"	"	"	"	"	"	---

LEGEND:

M. R. -----The Methyl Red test.
 VP. -----Voges-Proskauer test.
 Cit. -----Sodium citrate test.

The results of this table show that of the ninety-two strains of Escherichia coli carried into the special tests fifty-two (52%) showed the presence of non-fecal Escherichia coli. Of this number it is interesting to note that nineteen (63%) of the thirty cisterns tested contained non-fecal Escherichia coli. Thirty-three (43%) of the seventy-six wells tested showed the presence of non-fecal Escherichia coli.

Samples showing fecal Escherichia coli numbered forty. Eight of this total were isolated from cisterns, and thirty-two from wells.

It was shown that eighty-six per cent of the samples tested evidenced the presence of Escherichia coli. It was further demonstrated that ninety per cent of the cisterns showed the presence of Escherichia coli, while eighty-six per cent of the wells showed Escherichia coli strains.

One of the most outstanding facts evidenced by this work was that five (49%) of the fourteen wells that were negative in the presumptive tests, originated from a deep source, while only six (9%) of sixty-two shallow wells were free of this organism. This would indicate that deep well water contains less pollution than water of shallow wells. The lack of surface washing and the formation of deep rock strata probably accounts for this.

The physical conditions around many of the wells and cisterns tested would lead one to assume that those who use water in the rural districts show little concern for the contamination of their drinking water supply.

SUMMARY AND CONCLUSIONS

In this study seventy-six wells and thirty cisterns were examined for the presence of Escherichia coli and total bacterial colony count.

It was shown that eighty-six per cent of the samples tested evidence the presence of Escherichia coli. It was further demonstrated that ninety per cent of the cisterns showed the presence of Escherichia coli, while eighty-six per cent of the wells showed Escherichia coli strains.

One of the most outstanding facts evidenced by this work was that five (49%) of the fourteen wells that were negative in the presumptive tests, originated from a deep source, while only six (9%) of sixty-two shallow wells were free of this organism. This would indicate that deep well water contains less pollution than water of shallow wells. The lack of surface washing and the formation of deep rock strata probably accounts for this.

The physical conditions around many of the wells and cisterns tested would lead one to assume that those who use water in the rural districts show little concern for the contamination of their drinking water supply.

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Reaction combinations	Possible interpretation when isolated from water by the standard method	Common sources.
APPENDIX		
	USUALLY.	OCCASIONALLY.
APPLIES TO PURE STRAINS MEMBERS OF THE O-24 GROUP ONLY.		
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O	-	-
D R. P.	-	-
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Approved for Release by NSA on 09-11-2013 pursuant to E.O. 13526

COLI-AEROGENES GROUP---REACTION CLASSIFICATION¹⁰

Reaction combinations		Possible interpretation when isolated from water by the standard method		Common source.	
L O D N I	R. P. M. V.	E T A R T I C	USUALLY.	OCCASIONALLY.	APPLIES TO PURE STRAIN MEMBERS OF THE C-a GROUP ONLY.
/ / - -		Esch. coli	-----	Predominate in feces about 50 per cent of total group in sewage. Minority form in feces	
- / - -		" "	Non-members of group	Minority form in soil and sewage and feces	
/ / - /		Mixture	Intermediate strain sometimes considered non-typical Esch. coli	Soil, minority forms in sewage and feces	
- / - /		Intermediate strain	Mixtures or slow secondary reacting A. aerogenes	Soil, minority forms in sewage and feces	
/ / / /		Mixture	Atypical	Soil, sewage	
/ - - /		Always mixture			
/ - / /		Mixture	A. cloacae	Soil, minority forms in sewage and feces	
- / / /		A. aerogenes	Mixture	Majority forms in soil and on vegetables	
- - / /		" "		Up to 50 per cent of total group in sewage	
- - - /		Extraneous form	A. aerogenes	Minority form in feces	

¹⁰Standard Methods for Examination of Water and Sewage, Am. Pub. Health Assn., 1937 Ed. pp. 270

